



Original research

Determination of some pesticide residues in fresh fruits and vegetables using QuEChERS method followed by gas chromatography–mass spectrometry

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ABSTRACT

The fast-growing global concern of food safety has resulted in increased monitoring of pesticide residues in food products. In this study, 250 different samples of fruits (apple, orange) and vegetables (leafy vegetables, tomato, cucumber and lettuce) were collected from terminal markets in East Azerbaijan and Ardabil provinces of Iran and analyzed to investigate the presence of 23 widely used pesticide residues using gas chromatography equipped with mass spectrometry (GC–MS). The obtained results showed that some chemical contents of the evaluated samples exceeded the Codex maximum residue levels (MRLs). These chemicals included 2,4–DDT, malathion, bioallethrin, cypermethrin, carbaryl, diazinon, tebuconazole, and trifloxystrobin in leafy vegetables; diniconazole, bromopropylate, and chlorpyrifos in tomatoes; imazalil in oranges; phosmet, piperonyl–butoxide, and imazalil in cucumbers; propoxur in apples; and metalaxyl, bioallethrin, and diazinon in lettuces. These results play an alarm to consumers who are concerned about the pesticide residues in their foods.

Keywords: Fresh fruit; Vegetable; GC–MS; QuEChERS method; Pesticide residues

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1. Introduction

Pesticides are agrochemical substances formulated to prevent, control, and kill harmful pest organisms such as insects and fungi. Based on the action, pesticides are generally classified to insecticides (control insects), fungicides (control molds and fungi), herbicides (control weeds), and rodenticides (control rodents) (Sheridan & Meola, 1999). Pesticides are key components of agricultural technology due to food sustainable production and quality. However, extensive use of them can result in residues in foods with toxic side effects and serious health hazards (Lombardo, 1989).

The Environmental Protection Agency (EPA) has demonstrated that many pesticides are potential carcinogens (<https://www.epa.gov>). Therefore, indicating that the fresh and processed fruits and vegetables are free of pesticide residues is

absolutely necessary. Multiple factors have been shown to affect pesticide residues levels in the food products including plant surface texture, time interval, and frequency of pesticide application as well as weather conditions such as rainfall, temperature, humidity, sunlight, and wind (Nithya & Padmini, 2005). For example, vegetables are vulnerable to pests, especially in tropical regions, because in such regions the local climate facilitates the rapid reproduction of pests (Capinera, 2001). Since it is still unclear whether low levels of pesticides are safe or harmful for the consumers, governments limit pesticide residues in foods using the pesticide tolerance or maximum residue levels (MRLs) (Wing, 1994).

The increased public concern has resulted in the increased assessment and monitoring of pesticide residues in fresh and processed foods using routine methods. Recently, Association of Official Analytical Chemists (AOAC) has approved QuEChERS as an official pesticide analysis method in fruits and vegetables due to

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its high speed, easy operation, low cost, and safety (Ellison et al., 2009). This method is appropriate for the multiple-residue detection of almost all classes of pesticides. Furthermore, gas chromatography equipped with mass spectrometry (GC-MS) is a simple, rapid, cost-effective, and reliable method for the detection of trace levels of pesticide residues in foods (Nithya & Padmini, 2005).

The main goal of this study was to explore the potential application of QuEChERS method in combination with GC-MS for the extraction and determination of twenty-three pesticide residues belong to various chemical classes in different fresh fruit and vegetable samples consumed in the East Azerbaijan and Ardabil provinces of Iran. This method and the obtained results can contribute in the improvement of food quality as well as monitoring level of pesticide usage in the evaluated samples. Rapidity, simplicity in the operation, and environmental friendliness are the main advantages of the proposed method.

2. Material and Methods

2.1. Sample collection

In this study, totally 250 samples of six various types of fruits and vegetables were assessed. These samples were collected throughout the spring of 2017 from the East Azerbaijan and Ardabil provinces that cultivated in different regions of Iran. Samples were collected from terminal markets, transported to the laboratory and stored at 4 °C until the moment of analysis. Blank samples (pesticide-free samples) of the evaluated fresh fruits and vegetable samples were also obtained from small farms which were prepared in the East Azerbaijan and Ardabil provinces for control purposes. These samples were used as blanks in the quality assurance/quality control analysis.

2.2. Chemicals and standard solutions

Acetonitrile (ACN), ethyl acetate, anhydrous magnesium sulfate (MgSO_4), sodium chloride (NaCl), *tri*-sodium citrate dihydrate ($\text{Na}_3\text{Citrate}\cdot 2\text{H}_2\text{O}$), *di*-sodium hydrogen citrate sesquihydrate ($\text{Na}_2\text{HCitr}\cdot 1.5\text{H}_2\text{O}$), formic acid, primary secondary amine (PSA), and graphitized carbon black (GCB) were obtained from Merck (Darmstadt, Germany).

The twenty-three analytes belong to the various types of pesticides including insecticides (malathion, dichlorodiphenyltrichloroethane (2,4-DDT), chlorpyrifos, endosulfan-II, phosmet, 4,4-DDD, propoxur, lambda-cyhalothrin, endosulfan-sulfate, cypermethrin, diazinon, carbaryl, bioallethrin, piperonyl-butoxide, and pirimiphos-methyl), fungicides (tebuconazole, diniconazole, metalaxyl, trifloxystrobin, and imazalil), herbicides (thiobencarb), and acaricides (propargite and bromopropylate) were purchased from Sigma Aldrich, USA. Triphenylmethane was also supplied from Sigma-Aldrich and used as an internal standard (IS). A stock solution of the selected pesticides was prepared in acetonitrile with a concentration of $2500\ \mu\text{g}\cdot\text{mL}^{-1}$ (each pesticide). Working standard solutions were obtained daily by suitable stepwise dilution of the stock solution with acetonitrile and shaking them just prior to use. Also, a stock solution of internal standard was prepared by dissolving triphenylmethane in ethyl acetate at a concentration of $100\ \mu\text{g}\cdot\text{mL}^{-1}$. All of the solutions were stored at $-20\ ^\circ\text{C}$ until use.

2.3. Extraction and clean-up

A fresh fruit or vegetable sample (100 g in each case) was chopped, squeezed and homogenized by Buchi Mixer B 400 (Flawil, Switzerland). Then, a portion of it as a subsample (10 g) was taken and used in the following extraction procedure. Briefly, 10 mL of ACN were added to 10 g of homogenized samples in a 50-mL sterile centrifuge tube. This solution was shaken intensively for 1 min. Then, 4 g of MgSO_4 , 1 g of NaCl, 1 g of $\text{Na}_3\text{Citrate}\cdot 2\text{H}_2\text{O}$, and 0.5 g of $\text{Na}_2\text{HCitr}\cdot 1.5\text{H}_2\text{O}$ were added to the solution. Afterward, the tube was shaken vigorously for 2 min to prevent salt agglomeration before centrifugation at 3000 rpm for 10 min. One milliliter of the supernatant aliquot was added to a sterile centrifuge tube containing 25 mg of PSA, 150 mg of MgSO_4 , and 7.5 mg of GCB to remove pigments. The obtained mixture was shaken for 30 s and then centrifuged at 3000 rpm for 5 min. This was preserved using 5% formic acid in ACN and transferred to sterile vial for GC-MS analysis.

2.4. GC analysis of pesticide residues

The analysis instrument included a Benelux Joint Analytical System with an Agilent Technologies 7890A Network GC system. The MS included an Agilent Technologies 5975C Invert XL EI/CI MSD Selective Detector. The analysis column included Agilent 19091S-433 HP-5MS with 5% phenyl methyl siloxane; the autosampler included G2614 and the injector included Agilent Technologies 7683B series. The $30\ \text{m} \times 0.25\ \text{mm}$ i.d. capillary column with a $0.25\text{-}\mu\text{m}$ film was used in combination with the following oven temperature program: initially held at $75\ ^\circ\text{C}$ for 3 min, then programmed at a $25\ ^\circ\text{C}\cdot\text{min}^{-1}$ ramp to $150\ ^\circ\text{C}$, followed by a $5\ ^\circ\text{C}\cdot\text{min}^{-1}$ ramp to $300\ ^\circ\text{C}$, and held for 5 min. Helium with the purity of 99.9995% was used as the carrier gas at a constant flow rate of $1.0\ \text{mL}\cdot\text{min}^{-1}$. Splitless injection of $1\text{-}\mu\text{l}$ volume was carried out at $250\ ^\circ\text{C}$ with the purge valve on at 2 min. The MS interface temperature was maintained at oven temperature and operated in full scan mode with a range of $50\text{-}500\ m/z$. Samples were loaded into the MS instrument where evaporated and ionized by electron impact, resulting in positively charged particles which were then accelerated using an electric field. The mass-to-charge ratio (m/z) of the particles were computed based on the details of the motion of ions as they moved through the electromagnetic field and were detected and interpreted.

2.5. Analytical quality assurance/quality control (QA/QC)

Quantitative characteristics of the used method such as linear range (LR) of the calibration curves, limit of detection (LOD), limit of quantification (LOQ), precision, and recovery were determined to evaluate the method performance based on DG SANCO Guidelines (European Commission, 2011).

For the evaluation of linearity, matrix-matched calibration curves (plotting the ratios of analytes peak area to IS peak area versus the analytes concentration spiked to blank fresh fruit and vegetable samples) were prepared. According to the obtained results, good linearities are obtained with coefficient of determination higher than or equal to 0.9941 for all of the evaluated compounds (Table 1).

The LODs and LOQs were calculated at matrix-matched calibration standard as concentrations producing signal-to-noise ratios (S/N) of 3 and 10, respectively. Table 1 summarizes the

detection and quantification limits obtained for the individual pesticides studied. For each analyte, a concentration range is defined that relates to LODs and LOQs of analyte in different fresh fruit and vegetable matrices (the lowest and highest values were reported for each analyte).

Precision is defined as the nearness of the individual measurements of an analyte (measurement of the random error). Precision of the method, expressed as relative standard deviation (RSD), was assessed for both intra- and inter-day precisions. The precision was evaluated by performing the method on six quality control (QC) samples (for intra-day) and four QC samples (for inter-day) at a concentration of $30 \mu\text{g Kg}^{-1}$ of each analyte. All RSD% values were less than 5% (Table 1).

Other components of a sample matrix besides the analyte of interest could inhibit the extraction of an analyte or enhance its migration into the extraction solvent leading to biased quantification of the analyte. It is therefore essential to perform recovery studies to determine the applicability of proposed method in the presence of various sample matrices. For this purpose, the added-found method was used and the fresh fruit and vegetable samples were spiked with the analytes at two concentration levels (30 and $50 \mu\text{g Kg}^{-1}$ of each pesticide) and the method was performed on them (three times for each concentration). According to the obtained results, the mean percentage recoveries of studied pesticides in apple and orange samples ranged from 83 to 99 and 82 to 103%, respectively. In addition, mean percent recovery values of analytes were obtained between 83–101, 85–100, 81–104, and 83–104% for tomato, cucumber, leafy vegetables, and lettuce. In all cases RSD% values were less than 5%. These results are satisfactory (within the normal acceptable range of 70–120%) and

suggest that this method can be used for the determination of the evaluated analytes in the selected matrices.

3. Results and Discussion

Detected pesticides were classified as organochlorines, organophosphorus, carbamates, pyrethroids, and synergist insecticides, fungicides, acaricides, and herbicides based on the chemical classes (Table 1). Results of the sample analysis are summarized in Tables 2–7.

Pesticides detected in leafy vegetables (collected from Marand and cultivated in Isfahan) included diazinon (0.063 mg Kg^{-1}) and metalaxyl (0.18 mg Kg^{-1}) (Table 2). Concentration of diazinon was higher than that allowed by the Codex MRLs (0.05 mg Kg^{-1}) (FAO and WHO, 2003; 2005; 2007). In the case of leafy vegetables collected and cultivated in Tabriz diazinon (0.2 mg Kg^{-1}), tebuconazole (0.65 mg Kg^{-1}), trifloxystrobin (0.74 mg Kg^{-1}), piperonyl-butoxide (0.023 mg Kg^{-1}), bioallethrin (0.15 mg Kg^{-1}), bromopropylate (0.021 mg Kg^{-1}), propargite (0.018 mg Kg^{-1}), and cypermethrin (1.4 mg Kg^{-1}) were detected (Table 2). Concentrations of diazinon, tebuconazole, trifloxystrobin, bioallethrin, and cypermethrin exceeded the Codex MRLs (0.05 mg Kg^{-1}) (FAO and WHO, 2003; 2005; 2007). In samples collected from Ardabil and cultivated in Tehran 2,4-DDT, malathion, thiobencarb, pirimiphos-methyl, and diazinon (0.43 , 0.11 , 0.035 , 0.34 , and 0.15 – 0.82 mg Kg^{-1} , respectively) were detected (Table 2). Concentrations of 2,4-DDT, malathion, and diazinon were higher than those allowed by the Codex MRLs (0.05 mg Kg^{-1}) (FAO and WHO, 2003; 2005; 2007).

Table 1. Type, chemical group, and quantitative features of the pesticides selected for this study.

| Pesticide | Type | Chemical group | LOD ($\mu\text{g Kg}^{-1}$) | LOQ ($\mu\text{g Kg}^{-1}$) | LR ($\mu\text{g Kg}^{-1}$) | RSD%* | |
|--------------------|-------------|------------------|----------------------------------|----------------------------------|---------------------------------|-----------|-----------|
| | | | | | | Intra-day | Inter-day |
| Thiobencarb | Herbicide | Thiocarbamate | 7.7–8.2 | 23.3–24.8 | 23.3–300 | 2.1 | 2.9 |
| Malathion | Insecticide | Organophosphate | 5.3–6.5 | 16.9–20.7 | 16.9–350 | 3.3 | 4.1 |
| 2,4-DDT | Insecticide | Organochlorine | 3.2–3.9 | 10.5–12.8 | 10.5–400 | 2.4 | 3.5 |
| Chlorpyrifos | Insecticide | Organophosphate | 4.6–5.1 | 14.2–15.8 | 14.2–350 | 4.1 | 4.9 |
| Endosulfan-II | Insecticide | Organochlorine | 4.3–6.9 | 13.8–22.8 | 13.8–400 | 2.8 | 3.3 |
| Diniconazole | Fungicide | Triazole | 3.6–3.9 | 11.8–12.8 | 11.8–400 | 4.0 | 4.6 |
| Imazalil | Fungicide | Imidazole | 5.1–6.8 | 16.1–21.5 | 16.1–400 | 3.9 | 5.0 |
| Phosmet | Insecticide | Organophosphate | 5.9–7.6 | 18.8–24.2 | 18.8–350 | 3.7 | 4.2 |
| 4,4-DDD | Insecticide | Organochlorine | 3.3–6.2 | 10.5–19.8 | 10.5–400 | 2.2 | 4.6 |
| Propoxur | Insecticide | Carbamate | 6.6–7.9 | 20.6–24.8 | 20.6–350 | 3.1 | 4.5 |
| Lambda-cyhalothrin | Insecticide | Pyrethroid | 7.6–7.9 | 23.9–24.8 | 23.9–350 | 1.9 | 2.8 |
| Metalaxyl | Fungicide | Phenylamide | 8.6–8.9 | 26.6–27.6 | 26.6–400 | 2.4 | 3.9 |
| Trifloxystrobin | Fungicide | Strobilurin | 9.3–9.8 | 28.1–29.4 | 28.1–300 | 3.3 | 4.6 |
| Endosulfan-sulfate | Insecticide | Organochlorine | 8.3–9.0 | 26.5–28.7 | 26.5–350 | 4.1 | 4.9 |
| Tebuconazole | Fungicide | Triazole | 7.3–8.9 | 23.4–28.4 | 23.4–300 | 3.8 | 4.2 |
| Cypermethrin | Insecticide | Pyrethroid | 8.6–9.1 | 27.4–28.9 | 27.4–400 | 3.2 | 3.9 |
| Diazinon | Insecticide | Organophosphate | 3.1–3.9 | 10.2–12.8 | 10.2–400 | 2.7 | 3.2 |
| Carbaryl | Insecticide | Carbamate | 3.0–6.8 | 9.6–21.8 | 9.6–350 | 2.4 | 3.8 |
| Bioallethrin | Insecticide | Pyrethroid | 8.6–9.3 | 26.7–28.9 | 26.7–400 | 3.2 | 3.8 |
| Piperonyl-butoxide | Insecticide | synergist | 6.9–7.2 | 21.4–22.3 | 21.4–350 | 3.9 | 4.3 |
| Propargite | Acaricide | Sulphite ester | 8.6–9.3 | 26.6–28.8 | 26.6–300 | 3.8 | 4.0 |
| Bromopropylate | Acaricide | Benzilate | 7.6–8.0 | 23.5–24.9 | 23.5–350 | 4.3 | 4.8 |
| Pirimiphos-methyl | Insecticide | Phosphorothioate | 5.6–7.5 | 18.4–24.8 | 18.4–300 | 2.1 | 2.9 |

* In each case the highest value is reported.

Table 2. Concentrations (mg Kg⁻¹) of pesticides detected in the leafy vegetables in spring.

| Product | | Leafy vegetables | | | | |
|-----------------------|---------------|------------------|--------|--------|--------|---------|
| Region of sampling | | Ardabil | Marand | Mianeh | Tabriz | Marand |
| Region of cultivation | | Tehran | Tabriz | Tehran | Tabriz | Isfahan |
| 2,4-DDT | Concentration | 0.43 | ND | ND | ND | ND |
| | MRL | 0.05 | - | - | - | - |
| Malathion | Concentration | 0.11 | ND | ND | ND | ND |
| | MRL | 0.05 | - | - | - | - |
| Thiobencarb | Concentration | 0.035 | ND | ND | ND | ND |
| | MRL | 0.05 | - | - | - | - |
| Pirimiphos methyl | Concentration | 0.34 | ND | ND | ND | ND |
| | MRL | 0.05 | - | - | - | - |
| Bromopropylate | Concentration | ND | ND | ND | 0.021 | ND |
| | MRL | - | - | - | 0.05 | - |
| Propargite | Concentration | ND | ND | ND | 0.018 | ND |
| | MRL | - | - | - | 0.05 | - |
| Pipreonyl butoxide | Concentration | ND | ND | ND | 0.023 | ND |
| | MRL | - | - | - | 0.05 | - |
| Bioallethrin | Concentration | ND | ND | ND | 0.15 | ND |
| | MRL | - | - | - | 0.05 | - |
| Cypermethrin | Concentration | ND | ND | 0.26 | 1.4 | ND |
| | MRL | - | - | 0.05 | 0.05 | - |
| Carbaryl | Concentration | ND | ND | 0.13 | ND | ND |
| | MRL | - | - | 0.05 | - | - |
| Diazinon | Concentration | 0.15-0.82 | 0.12 | ND | 0.2 | 0.063 |
| | MRL | 0.05 | 0.05 | - | 0.05 | 0.05 |
| Cypermethrin-1 | Concentration | ND | ND | 0.26 | 0.061 | ND |
| | MRL | - | - | 0.05 | 0.05 | - |
| Tebuconazole | Concentration | ND | ND | ND | 0.65 | ND |
| | MRL | - | - | - | 0.05 | - |
| Trifloxystrobin | Concentration | ND | ND | ND | 0.74 | ND |
| | MRL | - | - | - | 0.05 | - |
| Metalaxyl | Concentration | ND | ND | ND | ND | 0.18 |
| | MRL | - | - | - | - | 2 |

ND: not detected.

Table 3. Concentrations (mg Kg⁻¹) of pesticides detected in the tomato samples in spring.

| Product | | Tomato |
|-----------------------|---------------|--------|
| Region of sampling | | Tabriz |
| Region of cultivation | | Tabriz |
| Bromopropylate | Concentration | 0.05 |
| | MRL | 1 |
| Diniconazole | Concentration | 0.068 |
| | MRL | 0.05 |
| Chlorpyrifos | Concentration | 0.42 |
| | MRL | 0.1 |

Pesticides detected in leafy vegetables collected from Mianeh and cultivated in Tehran included cypermethrin (0.26 mg Kg⁻¹) and carbaryl (0.13 mg Kg⁻¹) (Table 2). These concentrations exceeded the Codex MRLs (0.05 mg Kg⁻¹) (FAO and WHO, 2003; 2005; 2007). In the case of leafy vegetables that collected from Marand and cultivated in Tabriz, diazinon residues (0.12 mg Kg⁻¹) were detected that was higher than Codex MRLs (0.05 mg Kg⁻¹) (FAO and WHO, 2003; 2005; 2007). Mtashobya (2017) assessed the concentrations of pesticide residues in vegetables collected from Tanzania. Concentration of malathion was lower than that detected

in the current study (0.003 instead of 0.11 mg Kg⁻¹). In contrast, Li et al. (2014) studied pesticide residues in fresh vegetables from Heibei province, China, and reported the concentration of malathion in cole samples as 0.23 mg Kg⁻¹. This level was higher than that found in the current study. In total, 53.33% of investigated samples were rejected. Also, Osei-Fosu et al. (2014) reported that 20% of the vegetables from Agbogboshie exceeded the EU adopted values of MRLs.

Table 4. Concentrations (mg Kg⁻¹) of pesticides detected in the orange samples in spring.

| Product | | Orange | |
|-----------------------|---------------|--------|---------|
| Region of sampling | | Tabriz | Marand |
| Region of cultivation | | Tabriz | Isfahan |
| Diazinon | Concentration | 0.05 | ND |
| | MRL | 0.7 | 0.7 |
| Imazalil | Concentration | 0.24 | 0.072 |
| | MRL | 0.05 | 0.05 |

ND: not detected.

Table 5. Concentrations (mg Kg⁻¹) of pesticides detected in the cucumber samples in spring.

| Product | | Cucumber | | |
|-----------------------|---------------|----------|---------|---------|
| Region of sampling | | Tabriz | Ardabil | Marand |
| Region of cultivation | | Tabriz | Tehran | Isfahan |
| Chlorpyrifos | Concentration | 0.02 | ND | ND |
| | MRL | 0.05 | - | - |
| 2,4-DDT | Concentration | 0.02 | ND | ND |
| | MRL | 0.05 | - | - |
| 4,4-DDD | Concentration | 0.04 | ND | ND |
| | MRL | 0.05 | - | - |
| Malathion | Concentration | 0.024 | ND | ND |
| | MRL | 0.2 | - | - |
| Phosmet | Concentration | 0.15 | ND | ND |
| | MRL | 0.05 | - | - |
| Piperonyl butoxide | Concentration | - | 0.25 | ND |
| | MRL | - | 0.05 | - |
| Imazalil | Concentration | - | - | 0.072 |
| | MRL | - | - | 0.05 |

ND: not detected.

Table 6. Concentrations (mg Kg⁻¹) of pesticides detected in the apple samples in spring.

| Product | | Apple | | | |
|-----------------------|---------------|--------|--------|---------|----------|
| Region of sampling | | Tabriz | Mianeh | Ardabil | Maragheh |
| Region of cultivation | | Tabriz | Tehran | Tehran | Salmas |
| Chlorpyrifos | Concentration | 0.24 | 0.016 | ND | ND |
| | MRL | 1 | 1 | - | - |
| Propargite | Concentration | ND | ND | 0.38 | 0.5 |
| | MRL | - | - | 1 | 1 |
| Propoxur | Concentration | 0.07 | ND | ND | ND |
| | MRL | 0.05 | - | - | - |
| Bromopropylate | Concentration | ND | ND | ND | 0.3 |
| | MRL | - | - | - | 5 |

ND: not detected.

Table 7. Concentrations (mg Kg⁻¹) of pesticides detected in the lettuce samples in spring.

| Product | | Cucumber | | |
|-----------------------|---------------|----------|--------|---------|
| Region of sampling | | Tabriz | Mianeh | Marand |
| Region of cultivation | | Tabriz | Tehran | Varamin |
| Metalaxyl | Concentration | 0.067 | ND | ND |
| | MRL | 0.05 | - | - |
| Lambda-cyhalothrin | Concentration | ND | 0.025 | ND |
| | MRL | - | 0.05 | - |
| Bioallethrin | Concentration | 0.072 | 0.3 | ND |
| | MRL | 0.05 | 0.05 | - |
| Carbaryl | Concentration | ND | 0.034 | ND |
| | MRL | - | 0.05 | - |
| Diazinon | Concentration | ND | ND | 0.18 |
| | MRL | - | - | 0.05 |
| Piperonyl butoxide | Concentration | ND | ND | 0.05 |
| | MRL | - | - | 0.05 |

ND: not detected.

Pesticide residues in spring tomatoes collected and cultivated in Tabriz included bromopropylate, diniconazole, and chlorpyrifos (0.05, 0.068, and 0.42 mg Kg⁻¹, respectively) (Table 3). Concentrations of diniconazole and chlorpyrifos were higher than the eligible Codex MRL concentrations of 0.05 and 0.1 mg Kg⁻¹,

respectively (FAO and WHO, 2007). Totally, 5.26% of the evaluated tomato samples were rejected. Mtashobya (2017) investigated γ -HCH, α -HCH, and β -endosulfan pesticides in tomato samples from Uluguru during the rainy seasons. The predominant pesticides in the tomato samples from Accra

Metropolis, Ghana, included γ -HCH (lindane) and δ -HCH (Bempah & Donkor, 2011). Relatively, lindane, T-endosulfan, and DDT were detected in winter tomato samples from Agra, India, at the concentrations of 0.004 mg Kg⁻¹, 0.007 mg Kg⁻¹, and 0.005 mg Kg⁻¹, respectively (Bhanti & Taneja, 2005).

In the case of spring orange samples, imazalil (0.072 mg Kg⁻¹) was detected in the samples collected from Marand and cultivated in Isfahan (Table 4). In addition, diazinon (0.05 mg Kg⁻¹) and imazalil (0.24 mg Kg⁻¹) residues were found in the samples collected and cultivated in Tabriz. The mean level of imazalil in all samples exceeded the Codex MRLs (0.05 mg Kg⁻¹) (FAO and WHO, 2003) and 16.66% of the investigated samples were rejected.

In spring cucumber samples (collected from Marand and cultivated in Isfahan) imazalil was found at a concentration of 0.072 mg Kg⁻¹ that exceeded the Codex MRLs (0.05 mg Kg⁻¹) (Table 5) (FAO and WHO, 2007). Pesticide residues detected in samples collected and cultivated in Tabriz included phosmet (0.15 mg Kg⁻¹), 2,4-DDT (0.02 mg Kg⁻¹), 4,4-DDD (0.04 mg Kg⁻¹), malathion (0.024 mg Kg⁻¹), and chlorpyrifos (0.02 mg Kg⁻¹). The phosmet concentration was higher than that allowed by the Codex MRLs (0.05 mg Kg⁻¹) (FAO and WHO, 2003; 2005; 2007). In addition, piperonyl-butoxide (0.25 mg Kg⁻¹) was detected in samples collected from Ardabil and cultivated in Tehran, exceeding the Codex MRLs (FAO and WHO, 2003; 2005; 2007). Totally, 17.64% of the evaluated cucumber samples were rejected. Similarly, Osei-Fosu et al. (2014) detected chlorpyrifos and malathion in cucumber samples from Greater Accra and Eastern of Ghana, respectively. Concentrations of chlorpyrifos and malathion were reported as 0.07 and 0.04 mg Kg⁻¹ respectively, which were higher than those reported in the present study. Li et al. (2014) detected acephate at a concentration of 0.17 mg Kg⁻¹ in cucumber samples from Hebei province, China. (Also, a study in Agra, India, showed that lindane (0.004 mg Kg⁻¹), T-endosulfan (0.001 mg Kg⁻¹), and DDT (0.0009 mg Kg⁻¹) were in summer cucumbers (Bhanti & Taneja, 2005).

Pesticides detected in apple samples (collected from Maragheh and cultivated in Salmas) included propargite (0.5 mg Kg⁻¹) and bromopropylate (0.3 mg Kg⁻¹). These concentrations were lower than those allowed by the Codex MRLs (1 and 5 mg Kg⁻¹, respectively) (Table 6) (FAO and WHO, 2003; 2005; 2007). No pesticide residues were detected in samples from Ajabshir which cultivated in Urmia. Samples collected from Ardabil and cultivated in Tehran contains propargite at a concentration of 0.38 mg Kg⁻¹ that was lower than that allowed by the Codex MRLs (1 mg Kg⁻¹) (FAO and WHO, 2003; 2005; 2007). Chlorpyrifos was detected in samples collected from Mianeh and cultivated in Tehran at a concentration of 0.016 mg Kg⁻¹, that was lower than the concentration allowed by the Codex MRLs (1 mg Kg⁻¹) (FAO and WHO, 2003; 2005; 2007). Chlorpyrifos and propoxur were detected in samples collected and cultivated in Tabriz. Concentration of propoxur (0.07 mg Kg⁻¹) exceeded the Codex MRLs (FAO and WHO, 2003; 2005; 2007). In total, 6.66% of the investigated apple samples were rejected. The presence of lindane, heptachlor epoxide, endrin aldehyde, endrin ketone, and DDT/DDE were detected in the imported apples of Accra Metropolis, Ghana (Bempah & Donkor, 2011).

Pesticides detected in spring lettuce samples collected from Marand and cultivated in Varamin included diazinon and piperonyl-butoxide (Table 7). Concentration of diazinon (0.18 mg Kg⁻¹) exceeded the Codex MRLs (0.05 mg Kg⁻¹) (FAO and WHO, 2003; 2005; 2007). Lambda-cyhalothrin (0.025 mg Kg⁻¹),

bioallethrin (0.3 mg Kg⁻¹), and carbaryl (0.043 mg Kg⁻¹) were detected in the samples collected from Mianeh that were cultivated in Tehran. Concentration of bioallethrin was higher than that allowed by the Codex MRLs (0.05 mg Kg⁻¹) (FAO and WHO, 2003; 2005; 2007). Pesticide residues of metalaxyl and bioallethrin with respective concentrations of 0.067 and 0.072 mg Kg⁻¹ were detected in the samples collected and cultivated in Tabriz. These concentrations exceeded the Codex MRLs (0.05 mg Kg⁻¹) (FAO and WHO, 2003; 2005; 2007). Totally, 23.52% of the evaluated lettuce samples were rejected. Osei-Fosu et al. (2014) detected lambda-cyhalothrin and diazinon in lettuces from Greater Accra at concentrations of 0.33–0.90 and 0.05–0.08 mg Kg⁻¹, respectively.

3.1. Potential health risks associated with exposure to exceeded Codex MRLs pesticides

Frequent exposure to pesticides is linked to chronic health problems in humans as well as serious damages to the environment (Bertolote et al., 2006). As shown in Tables 2–7, in some cases the detected values of pesticides exceeded their residue concentrations that allowed by Codex MRLs. The residue concentration of 2,4-DDT, malathion, bioallethrin, cypermethrin, carbaryl, diazinon, tebuconazole, and trifloxystrobin in leafy vegetable samples; diniconazole, bromopropylate, and chlorpyrifos in tomato samples; imazalil in oranges; phosmet, piperonyl-butoxide, and imazalil in cucumber samples; propoxur in apple samples; and metalaxyl, bioallethrin, and diazinon in lettuce samples exceeded their residue concentrations that allowed by Codex MRLs. Briefly, 2,4-DDT is the best known chemical group of organochlorine insecticides. Organochlorine pesticides are nerve toxins, tending to bioaccumulate in fat tissues (lipophilic), persisting in environment and biomagnifying in food chains (Schiaivone et al., 2010). Organophosphates such as malathion, diazinon, and chlorpyrifos are organic insecticides. Organophosphates interrupt the central nervous system (CNS), inhibiting an important neurotransmitter called cholinesterase. This results in convulsion and possibly death at high doses. However, most of these chemicals are degraded in the environment. The acute toxicity of these chemicals varies widely. For example, parathion is highly toxic for humans while malathion is less toxic (Maxwell, 2009). Carbamates such as propoxur include a similar mechanism of action but with lower short-term toxicity in humans (Maxwell, 2009). Bioallethrin and cypermethrin are insecticides so called pyrethroids. These pesticides include a lower toxicity in humans and are easily degraded in the environment (Maxwell, 2009). Fungicides such as metalaxyl, trifloxystrobin, tebuconazole, diniconazole, and imazalil are used to control fungi. These pesticides can irritate human skin, eyes and throat (Freeman et al., 2004).

In the current study, however, concentrations of the rest detected pesticides were below the established tolerable standards in food samples, but long-term consumption of such vegetables and fruits may accumulate body receptors and hence results in chronic side effects in humans (Bhanti & Taneja, 2005).

4. Conclusion

One of the main health problems is the pesticide residues contamination of foods. Consumers are at the greatest risk of exposure to pesticides through contaminated foods. To avoid unwanted effects of the pesticides on human's health, food products must not contain pesticide residues over than the eligible

Codex MRLs. In summary, results of the current study indicated the pesticide residues of some vegetables and fruits consumed in East Azerbaijan and Ardabil provinces of Iran. In total, 53.33% of leafy vegetables, 5.26% of tomatoes, 16.66% of oranges, 17.64% of cucumbers, 6.66% of apples, and 23.52% of lettuces were rejected. However, further comprehensive studies are necessary to determine the pesticide residues in other regions of Iran.

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Conflict of interest

The authors declare that they have no conflict of interest.

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